Evidence for an Additional *Littorina* Species and a Summary of the Reproductive Biology of *Littorina* from California

BY

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(2 Text figures)

INTRODUCTION

Studies of the reproductive patterns of California littorinaceans revealed 2 morphologically distinct types of egg capsules produced by females identified as *Littorina scutulata* Gould, 1849. These egg capsules differed in their shape, dimensions, number of eggs per capsule and in the size and location of the hatching pore through which the veligers emerged. The developmental rate within the capsules was also found to differ between the 2 types. The occurrence of dimorphic penes among male *L. scutulata* further suggests that this taxon, as construed on the basis of shell morphology, is a composite of 2 morphologically similar species.

How significant are such differences in this group of snails? It has been shown by various authors, including Whipple (1965), Borkowski & Borkowski (1969), Rosewater (1970) and Bandel (1974), that egg capsule morphology is a discrete species character. Borkowski & Borkowski (*op. cit.*) further found that both shape and size of egg capsules were a "good species discriminator" for 3 *Littorina* species of a similar shell morphology that occur together on rocky intertidal shores in southeastern Florida. It is also evident from the studies of Rosewater (*op. cit.*) and Bandel (*op. cit.*) that the utility of egg capsule morphology as a species character can be extended to faunal provinces rich in littorine species. Differences in embryonic and larval development can similarly be cited as species-specific traits. In males, the morphology of the penis has also been successfully employed in delineating littorinid species by Abbott (1954), Whipple (*op. cit.*), Rosewater (*op. cit.*), as well as others.

Shells in littorinids, however, demonstrate greater variability than do egg capsules or soft parts. Struhsaker (1968) has reviewed several examples of intraspecific variation within the Littorinidae. Her experimental work has further indicated that, for at least one species, variation in shell sculpture has a genetic basis, while survivorship of the 2 morphs depends upon the environment. Therefore, asynchronous environmental changes along a shoreline may serve to encourage variability among shells in a population. However, it is equally probable that a given environment may encourage convergence of shell characters of co-occurring species. The second hypothesis would serve to explain how 2 species might be considered as a single highly variable one, as present evidence suggests is the case for *Littorina scutulata*.

METHODS

Adult specimens of *Littorina scutulata* were collected from a variety of intertidal habitats from San Diego, California, U. S. A., north to Vancouver Island, British Columbia, Canada. Snails were packed damp in plastic bags, and hand-carried or air mailed in padded mailing bags. Specimens thus could be obtained throughout much of the species' range within 1 to 7 days without significant mortality. Upon arrival, snails were placed in Carolina Stacking Culture dishes (inside diameter 10.5 cm), half filled with filtered sea water at 15°C. The dishes of snails were then placed in a water bath of the same temperature and checked daily for egg capsules. Water was changed daily. Egg capsules were sorted on the basis of capsule shape and transferred to Stender dishes (inside diameter 5.3 cm) half filled with filtered sea water. Egg capsules were examined at 24-hour intervals until hatching. Following hatching, each egg capsule was examined and

point of hatching noted. The diameters of the hatching pore and of the capsule surface containing the pore were measured.

Individual spawning records, for fecundity estimates, were obtained by placing individual snails in the Stender dishes half filled with water and recording the presence or absence of egg capsules at 24-hour intervals. If spawning did not occur within 7 days, that snail was replaced; otherwise, snails were maintained until egg capsule production ceased. The number of eggs per capsule was recorded for all capsules produced in the laboratory.

This study was conducted at the Pacific Marine Station, Dillon Beach, California, from April until August 1973, and was continued at the Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island, during August 1974.

EGG CAPSULES AND FECUNDITY

The egg capsules produced by *Littorina* from California are planktonic. Within these capsules, the embryos develop to veligers before hatching. The number of eggs, and hence embryos, per capsule varies with species. In *L. planaxis* Philippi, 1847, no more than a single egg per capsule has been found in the several thousand examined. The number of eggs per capsule in *L. scutulata,* however, shows variability among capsules. This variation is stable for each capsule type, if either the range or mean of the number of eggs per capsule is considered. The differences in egg capsule morphology between the 2 *L. scutulata* morphotypes are described below.

Littorina scutulata, Type I: These egg capsules (Figure 1A) are slightly greater than 1 mm in maximum diameter. They consist of 2 biconcave discs separated by a central chamber containing the eggs. The overall appearance resembles that of an automobile wheel. The number of eggs contained in these capsules may range from 4 to 41 eggs, although 17 to 32 is usual. Variation in number of eggs per capsule is summarized in Table 1 for the stations studied.

The salmon-colored eggs prior to the first cleavage are 95.7μm in diameter. Each egg is enclosed within a transparent membrane 116.5μm in diameter. Fecundity estimates were not made for this group of *L. scutulata.*

Littorina scutulata Type II: The egg capsules described by BUCKLAND-NICKS *et al.* (1973), for *L. scutulata* are of this type. These capsules (Figure 1B) are shaped like inverted saucers and may contain 1 to 14 embryos, although 3 to 10 is usual (see Table 1). The egg capsule is slightly smaller than the Type I and contains fewer eggs. The eggs are salmon-colored, 105μm in diameter and contained within an egg membrane 130.5μm in diameter.

Table 1

The range and mean of the number of eggs per capsule spawned by Type I and II *Littorina scutulata* during 1973.

Source	Approximate N. Latitude	Type I range	Type I mean	Type II range	Type II mean
Ucluelet, British Columbia	49°00′	—	—	1-4	2.9
Anacortes, Washington	48°30′	—	—	present	
Newport, Oregon	44°40′	4-35	17.3	—	—
Howard Creek, Mendocino Cty. California	39°45′	—	—	1-14	10.4
Sea Ranch, California	38°42′	14-35	25.3	7-9	7.9
Dillon Beach California	38°10′	5-34	22.6	—	—
San Luis Obispo, California	35°25′	present		3-13	7.3
Pismo Beach, California	35°10′	10-28	20.8	—	—
Gaviota, California	34°25′	6-41	23.8	3-6	4.4
S. Laguna Beach, California	33°32′	19-40	32.8	3-12	7.6
San Diego California	32°45′	7-32	18.0	2-11	4.2

Both Type I and Type II capsules are readily distinguished from the capsules of *Littorina planaxis* by size, number of eggs and capsule morphology; see Figure 1 and Table 3.

Fecundity estimates for isolated Type II *Littorina scutulata* from central and northern California indicate a lower egg production and longer period of spawning than reported by BUCKLAND-NICKS *et al.* (1973), for Puget Sound, Washington. They report that 3 females produced 896, 1034 and 1398 egg capsules within a 2-week period before capsule production ceased. These capsules contained from 3 to 4 eggs each so that total egg production per female was between 9 900 and 13 300. In the present study, 4 females from Mendocino County, California, isolated in 1973, produced 156 to 235 capsules with a combined egg production of 7 220 eggs. Normalizing these data to a per-snail fecundity, the California snails were only half as fecund as the individuals from Puget Sound, although spawning lasted for 4 weeks with-

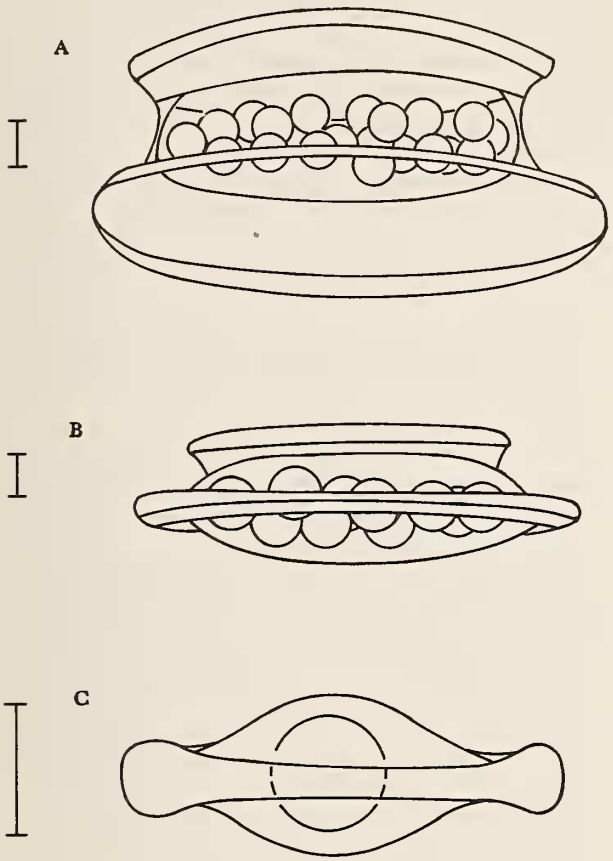

Figure 1

Pelagic egg capsules of California species of *Littorina*

A) *Littorina scutulata* Type I, hatching pore, through which veligers emerge, forms on the lower capsule surface
B) *Littorina scutulata* Type II, hatching pore forms on the upper capsule surface
C) *Littorina planaxis* Scale is 100 μm

out reinseminating the females, 2 weeks longer than the Puget Sound snails. In 1974, 31 females were collected at the tip of Bodega Head, Bodega Bay, California, and isolated for fecundity estimates. The highest capsule production by a single snail was 872. These capsules contained 6024 eggs. A second snail produced 842 capsules containing 6807 eggs. The maximum length of a single spawning period for this group of snails was 2 weeks.

It appears, then, that California *Littorina scutulata* (Type II) produce fewer eggs and capsules while investing more eggs in each capsule than do Puget Sound snails. With the data presently available, it is not possible to determine the source of differences in production. They may relate to population differences or to previous spawning history.

DEVELOPMENT AND HATCHING

In *Littorina scutulata,* Types I and II, the early development is synchronous (Table 2); the blastula forms in 24 hours, and the gastrula within 48 hours – 24 hours slower than in *L. planaxis*. The trochophore is reached in 4 days and from this point until hatching, development is asynchronous. In *L. scutulata,* Type I, the trochophore lasts for 24 hours before developing into a shelled veliger stage by day 5. In *L. scutulata,* Type II, the trochophore stage lasts 48 hours before developing into a shelled veliger on day 6. In both types, the veliger remains in the egg capsule for 2 days, emerging on day 7 in Type I and on day 8 in Type II. The developmental sequences of *L. planaxis* and the 2 *L. scutulata* are contrasted in Table 2.

Table 2

Summary of developmental events occurring within the egg capsule of California *Littorina* maintained at 15°C.

Day	*Littorina planaxis*	*Littorina scutulata* "Type I"	*Littorina scutulata* "Type II"
1	spawning	spawning	spawning
2	gastrula	blastula	blastula
3	trochophore	gastrula	gastrula
4	trochophore	trochophore	trochophore
5	veliger	veliger	trochophore
6	hatching	veliger	veliger
7		hatching	veliger
8			hatching

Hatching in *Littorina scutulata s. l.* is similar to that of *Lacuna pallidula* (da Costa, 1778) and *L. saxatilis* (= *L. rudis*) (Olivi, 1792) as reviewed by Davis (1968), in that the larvae emerge through a "hole." It is doubtful, however, that the "holes" are homologous to the hatching pores in *L. scutulata s. l. Lacuna pallidula* and *Littorina saxatilis* spend a proportionately longer developmental period in the egg mass, emerging as juveniles after rasping through their respective encasements with their radulae.

Littorina scutulata, in contrast, emerge as veligers at a predetermined site, the hatching pore. The pore is centrally located on one surface of the egg capsule and the larvae are incapable of emerging at any other point. This has been demonstrated by noting the fate of embryos in capsules with opposite disc surfaces lying against the bottom of the culture dish. In these capsules, the hatching pore, which is not visible prior to its completion, forms on the same surface, irrespective of orientation. Larvae in capsules whose hatching pore is adjacent to the bottom of the dish are unable to emerge although the pore is completely open.

The hatching pore in Type I *Littorina scutulata* is located on the capsule surface with the larger rim. It has a mean diameter of 250μm and represents an opening through which 2 larvae could simultaneously emerge. In Type II *L. scutulata,* the pore is located on the capsule surface with the smaller diameter and has a mean diameter of 340μm. This represents an opening through which 4 larvae could simultaneously emerge.

Rupture of the egg membrane is the first step in hatching and is apparently accomplished through a change in osmotic pressure. In both Type I and Type II *Littorina scutulata,* the egg membrane swells noticeably. Buckland-Nicks *et al.* (1973) reported a $\frac{1}{3}$ increase in egg membrane diameter prior to hatching. The egg membrane can swell as much as 7 to 9 times the initial volume before hatching. The veligers emerge from their egg membranes before the hatching pore is evident, and may be actively involved in the formation of this opening. Movement within the capsule is limited but some swimming occurs. Abrasion of the inner capsule wall by the shell, employment of the snail's radula and release of lytic enzymes from the larvae or egg membrane fluid may contribute to the emergence process. It also seems probable that the capsule wall is qualitatively different in the vicinity of the hatching pore or the point of emergence would be more variable. The presence of such a predetermined hatching pore is widely scattered through the mesogastropoda, having been previously reported in *Bithynia* (Hydrobiidae), *Lamellaria* (Lamellariidae) and *Trivia* (Eratoidae) by Fretter & Graham (1962); in *Strombus* (Strombidae) by D'Asaro (1965) and in *Monoplex, Mayena* and *Cabestana* (Cymatiidae) by Laxton (1969). It is not previously known from Littorinaceans.

DISTRIBUTION OF CAPSULE TYPES AND PENIS MORPHOLOGY

The area of coastline over which spawning females were obtained is roughly equivalent to the central third of the range reported by Oldroyd (1927) for *Littorina scutulata.* The type of capsule produced did not correlate with wave exposure or the nature of the substrate (rock, sand, muddy sand). Furthermore, females producing the 2 morphotypic capsules were found to co-occur at 5 of the California stations. The ranges of these morphotypes differ, however. Type I capsules were found only as far north as Newport, Oregon, while Type II capsules were found over the entire study area. Buckland-Nicks *et al.* (1973) did not record dimorphic capsules during their study at False Bay, Friday Harbor, Washington, nor were they present at the 2 stations reported here which bracket their study area. Based upon the absence of Type I capsules from Puget Sound to date, we may ascribe the capsule figured by Buckland-Nicks *et al.* (*op. cit.*), here designated as Type II, to *L. scutulata sensu stricto* because the area studied by these authors is the type locality for this species (Oldroyd, *op. cit.*).

Table 3

Summary of the reproductive biology of *Littorina* from California.

	Littorina scutulata Type I	*Littorina scutulata* Type 11	*Littorina planaxis*
Type of capsule	pelagic	pelagic	pelagic
Egg capsule diameter (mm)	1.1	0.7-1.0	0.3-0.4
Egg membrane diameter (μ)	116.5	130.5	100
Egg diameter (μ)	95.7	105	89
Number eggs per capsule	4-41	1-14	1
Number of days to hatching (15°C.)	7	8	6
Status at hatching	planktotrophic veliger	planktotrophic veliger	planktotrophic veliger
Shell width at hatching (μ)	169	155	137

A dimorphism in the penis of *Littorina scutulata* was also found (Figure 2). Considering only gross morphological detail, the form shown in Figure 2A has a conspicuous sperm groove running dorsally to a sub-terminal bulge. This form also possesses more hyaline granules than the second form and shows an area of canals with black pigmented borders where the penis inserts on the head. The penis in Figure 2B differs in the placement of the sperm groove, which runs laterally to the tip, and in possessing a large papilla on the dorso-lateral surface proximal to the curvature of the penis. No intermediate penis morphologies were observed.

The penes depicted in Figure 2A and B are both found in populations producing a mixture of the 2 egg capsule types. However, in a population from Anacortes, Washington (collected 13 May 1978 by Dr. A. J. Kohn) only the penis type shown in Figure 2A was found (15 males of 50 snails examined; sex ratio not significantly different from 2:1). The absence of dimorphic penes from Puget Sound *Littorina scutulata* therefore contributes additional evidence that populations in this area are monotypic. Following the reasoning used for assigning the identity of egg capsules, *L. scutulata s. s.* males are characterized by the penis morphology depicted in Figure 2A. The species heretofore considered to be *L. scutulata* is characterized by males possessing the penis type illustrated in Figure 2B and by females producing egg capsules like that shown in Figure 1A (Type I).

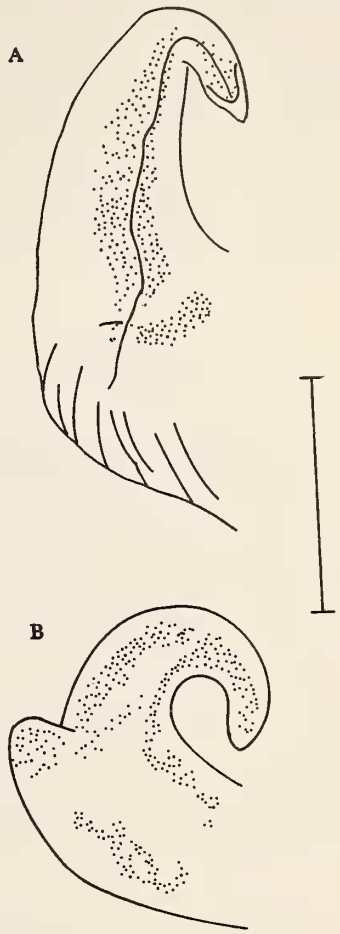

Figure 2

Littorina scutulata penis types. Scale is 1.0 mm

CONCLUSIONS

Based upon the differences in egg capsule morphology, developmental rates and penes between the 2 morphotypes of *Littorina scutulata*, it appears that *L. scutulata* is a complex of 2 species. Type II female snails are considered to belong to *L. scutulata sensu strico* as are males with the penis morphology depicted in Figure 2A. The identity of *L. scutulata* Type I, however, is uncertain. Gould (1849) described 2 additional species of *Littorina* from the Pacific Northwest, *L. plena* from San Francisco and *L. lepida* from Vancouver Island. A preliminary comparison of idiotypes of *L. plena* (MCZ 169298) and syntypes of *L. lepida* (MCZ 169222) indicates shell characters within the range of variation seen among female *L. scutulata* of both Types I and II. Therefore, *L. scutulata* Type I may be one of 2 species previously described by Gould or an as yet undescribed species which can co-occur wih *L. scutulata s. s.* from at least Newport, Oregon south to San Diego, California. The co-occurrence, the tendency towards variable shell characters and the lack of additional material precludes a complete delineation of species characteristics at present. However, it is reasonable to expect that differences in radulae also exist and that radular differences can be correlated with egg capsules produced by a female or with penes in males. The difficulty with shell characters arises from the diversity of habitats these species occupy and the well known influence of habitat upon molluscan shell characters. It is

hoped that once *L. scutulata* is recognized as a complex of 2 species, differences in shell morphology and geometry independent of habitat can be identified. Gould used several parameters in his descriptions that can be quantified, including shell length, width, apical and basal angles, and aperture shape. It should be possible using multivariate statistics (*e. g.*, principal components, discriminant function analysis, etc.) to separate habitat variability from variation between the 2 species at present regarded as *Littorina scutulata.*

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